for the unsymmetric substituted derivatives, such as styrene oxide, styrenimine, and phenylcyclopropane, it will be interesting to see if any degree of generality exists for this type of relation. There should be different ^{13}C–H couplings in the three-membered ring of these latter derivatives for each C–H bond.[41]

Conclusion

In the foregoing analysis we have attempted to demonstrate for one series of molecules containing a three-membered ring that there appears to exist a very simple linear relation between geminal and vicinal proton–proton couplings and the Huggins electronegativity[35] (or a related quantity derived from ^{13}C–H couplings) and that this relation is better than previous similar correlations would lead one to believe. The reason for the scatter in the previously offered correlations is the fact that certain substituents possess electronic interactions, which we shall term "extra electronic effects," whose presence does not contribute measurably to the determination of bond energies from which electronegativities are derived, but whose presence does provide additional possibilities for the propagation of nuclear spin–spin coupling. This may mean that in *many cases the ^{13}C–H coupling does not directly measure the percentage s character in a carbon bonding orbital when a carbon atom is substituted by one of the type of substituents capable of "extra electronic*

effects." This same conclusion has also been reached by Karabatsos,[42] who suggests that the breakdown of the simple correlation stems from spin–dipole and/or electron-orbital contributions to the ^{13}C–H coupling. However, Pople's recent theoretical treatment of the coupling between directly bonded atoms suggests that these two contributions will both be zero if one of two coupled nuclei is hydrogen.[43] On this basis it would seem that the deviations from a simple correlation of ^{13}C–H coupling with s character observed may well arise through a Fermi contact mechanism involving electrons of substituent atoms or groups. A theoretical consideration of this possibility could be fruitful.

Deviation from a simple correlation can probably *a priori* be expected for substituents with π-electrons, second and higher row elements, and fluorine atoms in close proximity to a set of coupled nuclei. Possibly low-lying excited states and/or d-orbital participation contribute to these effects for second and higher row elements. The nature of these electronic effects in the case of fluorine appears rather obscure.

The manner in which the relation between coupling constants and electronegativity has been discussed here could serve as a basis for making a separation between contributions to spin–spin coupling due to factors depending on bond strength and those derived from several extra electronic effects; these points are being further investigated.

(41) See R. M. Lynden-Bell, *Mol. Phys.*, 6, 537 (1963), for the case of vinyl bromide-^{13}C.

(42) G. J. Karabatsos and C. E. Orzech, *J. Am. Chem. Soc.*, 86, 3575 (1964).
(43) J. A. Pople and D. P. Santry, *Mol. Phys.*, 8, 1 (1964).

Carbon-13 Magnetic Resonance. III.[1] Purine

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Using deuterated compounds of known structure the carbon-13 chemical shift values for the 2, 6, and 8 carbons in purine were assigned unequivocally. The two remaining peaks found at the lowest and highest field positions are assigned to carbons 4 and 5, respectively, on the strength of the theoretical prediction that the highest π-electron density is at C-5. For an increasing magnetic field the resonance pattern for carbon-13 (2, 8, 6) differs from that noted for the corresponding directly bonded hydrogens (6, 2, 8). This disagreement suggests that chemical shift values should be used with care as a means for predicting charge densities. Nevertheless, a gross correlation of carbon-13 chemical shift data with theoretical estimates of the charge distribution in purine does exist.

I. Introduction

The recent literature contains several papers[2–6] in which the proton magnetic resonance spectra of

purine and some of its derivatives are discussed. Early assignments of the chemical shifts of the 2, 6, and 8 hydrogens in purine were based upon values obtained for purine derivatives and related heterocyclic compounds. More recent work has shown that these early assignments were in error, and the correct assignment has been obtained from various deuterium-substituted purines of known structure. Comparison of spectra of deuterated and nondeuterated species has made it possible to assign the peaks for an increasing field to the 6, 2, and 8 hydrogens, respectively.

In a series of aromatic compounds, Spiesecke and Schneider[7] have observed a direct relationship between the carbon-13 chemical shift value and the π-electron charge density. Their work has been given a theoretical

(1) Previous paper in series: D. M. Grant and E. G. Paul, *J. Am. Chem. Soc.*, 86, 2984 (1964).

(2) C. D. Jardetzky and O. Jardetzky, *ibid.*, 82, 222 (1960).
(3) S. Matsuura and T. Goto, *Tetrahedron Letters*, 1499 (1963).
(4) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, 86, 696 (1964).
(5) F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, 29, 1988 (1964).
(6) S. I. Chan, M.P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *J. Am. Chem. Soc.*, 86, 4182 (1964).
(7) H. Spiesecke and W. G. Schneider, *Tetrahedron Letters*, 14, 468 (1961).

basis by Karplus and Pople[8] who have shown that variations in estimates of charge densities and related free valence parameters adaquately account for the observed chemical shifts. In an extension of this treatment to a series of azines and methyl azines, Lauterbur[9] has shown that the Karplus–Pople formulation also applies to aromatic nitrogen heterocyclics. While theoretical estimates of charge densities in these compounds are not accurate enough to delineate completely the relative importance of σ- and π-electronic effects, the gross features have been correlated satisfactorily with this approach.

Several investigators[10–15] have calculated electron densities in purine. Because of the various assumptions made a wide range of predicted electron densities now exist in the literature. Coller[15] has explored the effect of introducing a variable electronegativity parameter into the calculations. One of the problems inherent in all of these calculations concerns the assignment of the labile proton in the imidazole ring to N-7 or N-9. In general the proton is believed to be at N-9, but no direct evidence has been presented to exclude the assignment on N-7. In their treatments, both Pullman and Pullman[11] and Coller[15] have considered both of the two alternative assignments in which the labile hydrogen in purine is placed at N-7 and N-9, respectively. Pullman and Pullman[11] have also treated the case in which the effect of the proton is averaged equally between both the N-7 and N-9 positions by assuming free exchange with the solvent. Our experimental data are compared with these calculated charge densities.

II. Experimental

A. Equipment. A Varian high-resolution spectrometer equipped with a V-4311 transmitter operating at 15.1 Mc.p.s. was used to observe the carbon-13 magnetic resonance spectra. Proton decoupling was accomplished with a Varian V-4320 spin decoupler operating at 60 Mc.p.s. The Varian V-4331 probe used in this work can be fitted either with a standard 5-mm. insert and Varian spinner attachment or with a 15-mm. insert which can be used for both 12- and 15-mm. sample tubes. The 12-mm. samples are spun with a Wilmad spinner assembly, whereas no provision is made for spinning the 15-mm. tubes.

The frequency of both the transmitter and the proton decoupler are measured to ± 1 c.p.s. with a Hewlett-Packard 524C electronic counter equipped with a 525A frequency converter. Chemical shift data are obtained by continuously monitoring the decoupler frequency as the decoupler setting is varied in accordance with the method described by Paul and Grant.[16] Because of its inherent stability, the frequency of the transmitter need be checked only oc-

(8) M. Karplus and J. A. Pople, *J. Chem. Phys.*, **38**, 2803 (1963).
(9) P. C. Lauterbur, private communication.
(10) T. Nakajima and B. Pullman, *J. Am. Chem. Soc.*, **81**, 3876 (1959).
(11) A. Pullman and B. Pullman, *Bull. soc. chim. France*, 766 (1958).
(12) B. Pullman, *J. Chem. Soc.*, 1621 (1959).
(13) S. F. Mason in "The Chemistry and Biology of Purines," a CIBA Foundation Symposium, G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1957, p. 72.
(14) R. L. Miller and P. G. Lykos, *Tetrahedron Letters*, **11**, 493 (1962).
(15) B. A. W. Coller, Ph.D. Thesis, University of Melbourne, Melbourne, Australia, 1963. See also R. D. Brown, *Proc. Roy. Australian Chem. Inst.*, **31**, 1 (1964).
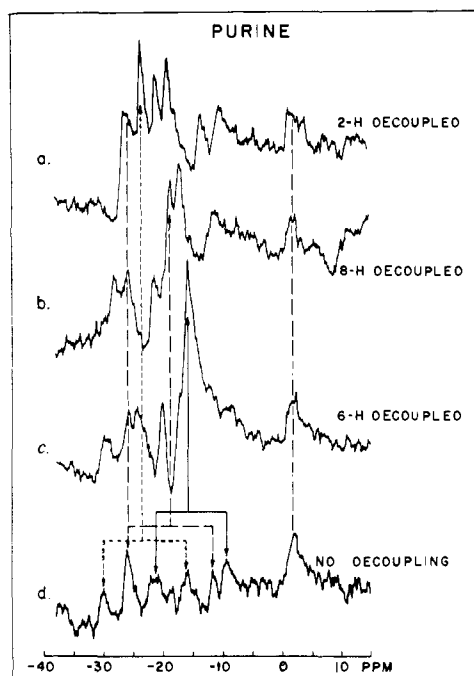(16) E. G. Paul and D. M. Grant, *J. Am. Chem. Soc.*, **86**, 2977 (1964).

casionally to ensure that this frequency is constant. Careful control of the ambient temperature is required if the required stability is to be achieved.

B. Spectroscopic Details. Purine samples at a concentration of 30% were prepared with water which had been boiled and degassed with oxygen-free nitrogen. The tubes then were sealed under vacuum. The decoupled carbon-13 resonance peaks were obtained[7] under both conditions of adiabatic rapid passage and of slow passage with spinning. The approximate decoupler frequency is determined under rapid passage conditions, and then with slow sweep and spinning a precise determination is made of the decoupling frequency from which the chemical shift is determined.

C. Synthesis of Deuteriopurines. 8-Deuteriopurine was prepared by heating purine in refluxing deuterium oxide for 4 hr. according to the procedure of Schweizer, *et al.*[4] Purification was by vacuum sublimation and recrystallization from toluene containing a small amount of absolute ethanol. 6,8-Dideuteriopurine was prepared and purified in a like manner by extending the refluxing time to 72 hr. Within experimental error this product was deuterated completely in position 8 and 80% deuterated in position 6 as judged from the p.m.r. spectra. The yield of 6,8-dideuteriopurine was approximately 20% since prolonged treatment of purine in refluxing deuterium oxide resulted in degradation of the compound.

III. Results

Considerable difficulty is encountered in obtaining carbon-13 resonance signals of a suitable signal to noise ratio in 30% purine solutions. We find some improvement in the signal heights when power is supplied to the sample at a frequency approaching the proton decoupling frequencies even though the doublets have not begun to coalesce. This magnetic perturbation of the sample to give slightly enhanced signals even though no coalescence is observed in the multiplet structure, is attributed to a nuclear Overhauser effect. The phenomenon is most beneficial in securing improved rapid passage spectra.

Originally, solutions of purine with a concentration of 37 and 45% by weight were studied. It was felt that a maximum concentration would lead to a better signal-to-noise ratio. However, owing to line broadening at higher concentrations, the peak heights actually decreased. A possible explanation of this effect is found in the strong intermolecular interactions postulated by Chan, *et al.*,[6] to account for changes in osmotic pressure and in p.m.r. chemical shift values as purine solutions are concentrated. They proposed that the planar purine molecules stack as platelets in very concentrated solutions. It is suggested that the molecular association is due to π-electron interactions between the purine rings. Dilution of the purine samples to about 30% results in narrower peaks and more favorable spectral conditions.

As an aid to the interpretation of the undecoupled, rapid passage spectrum of purine presented in Figure 1d, the frequency of the proton decoupler was varied through the proton resonance region of the 2, 6, and 8 hydrogens to eliminate the induced doublet structure in the corresponding carbon-13 spectrum. The constant

Figure 1. Adiabatic rapid passage C¹³ spectra at 15.1 Mc.p.s. of purine with proton decoupling. Chemical shift values in p.p.m. are relative to the benzene resonance peak.

Figure 2. Adiabatic rapid passage C¹³ spectra at 15.1 Mc.p.s. of two deuterated purines. Spectral peaks are not observed for carbons directly bonded to deuteriums.

positions occupied by the two singlet transitions at −25.9 p.p.m. and +0.4 p.p.m. from carbons 4 and 5 are easily recognized from these spectra. The assignment of the low-field peak to C-4 and the high-field peak to the fifth carbon rests on the theoretical prediction that the highest electron density is centered on C-5. Unequivocal assignment must await the synthesis of a purine labeled with carbon-13 in either position 4 or 5. These two singlets having a constant separation in Figure 1 provide helpful fiducial marks for analyzing the remaining spectral lines. The extensive overlapping of the proton induced doublets resulting from carbons 2, 6, and 8 prevented at first a clear assignment of the remaining three chemical shift values. As the six doublet lines move under the perturbing influence of the proton decoupler, strong lines are observed in the decoupled spectra which result not only from the complete collapse of a given doublet, but also from a coalescence of two or more lines from different multiplets. However, only those decoupled frequencies which collapse true doublet pairs of lines are found to give peaks under slow sweep conditions, whereas pseudo-peaks vanish because of saturation effects. Strong decoupled peaks are easily recognized in Figure 1 at the midpoint of doublets assigned to C-2 and C-6. When the 8 hydrogen is decoupled, the true C-8 peak appearing at the midpoint of this doublet is not readily recognized due to significant overlapping of several lines in this region of the spectrum. Furthermore, the downfield line of the C-8 doublet in the undecoupled spectrum (Figure 1d) is obscured by the low-field singlet assigned to C-4.

The assignment of the chemical shifts of the three doublets found at −23.1, −19.0, and −15.9 p.p.m. to C-2, C-8, and C-6, respectively, is made on the basis of the spectra contained in Figure 2 of deuterium-labeled purines. For the 6,8-dideuteriopurine only the
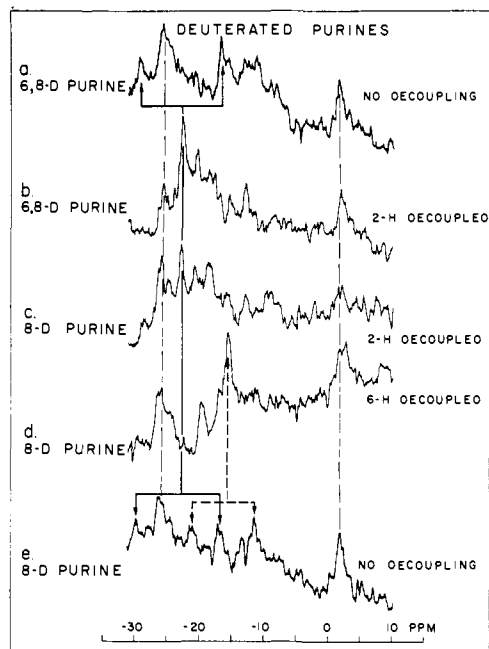
C-2 doublet and its corresponding proton-decoupled peak are observed as shown in Figures 2a and 2b. Resonance peaks for C-6 and C-8 are not observed due to deuterium substitution. Quadrupole broadening probably accounts for the elimination of these parts of the spectrum. Both the C-2 and C-6 spectral features were observed in the 8-deuteriopurine as shown in Figures 2c–2e. On this basis C-2 and C-6 were assigned directly, and by default the C-8 chemical shift was determined as it is the only remaining position to be assigned.

The spectrum given in Figure 3 is typical and represents an accumulation of all the slow sweep data taken on purine. Here the decoupler was continuously readjusted during the period of a single sweep in such a manner that a completely decoupled spectrum was obtained showing all five singlets arising from the five chemically different carbon atoms in purine. By using the chemical shifts of C-2, C-8, and C-6 which were determined accurately by the intensity plot method,[16] an accurate calibration of the sweep rate is obtained from which the chemical shifts of C-4 and C-5 were determined with reasonable accuracy. These values along with the proton chemical shift values measured in this laboratory are present in Table I.

An enhancement of the C-4 and C-5 singlets was observed for the setting of the decoupler given in Table I at the indicated transmitter frequency. This signal enhancement is attributed to the elimination of small proton–carbon-13 spin–spin interactions in the C-4 and C-5 singlets. Using the carbon-13 chemical shift values obtained from the calibrated sweep rate along with the decoupler frequencies required to enhance the C-4 and C-5 peaks, it is possible to calculate the position upon which the proton decoupler power was centered and these values in p.p.m. from TMS are given in parentheses in Table I. The position of decoupler power is within experimental error of the
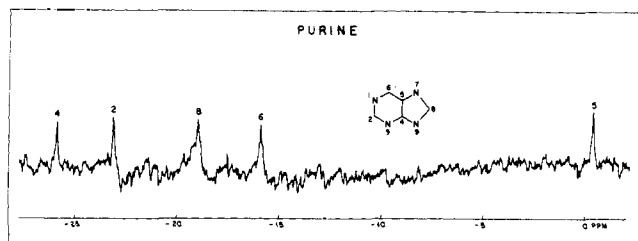
Figure 3. Slow sweep C^{13} spectrum of purine with proton decoupling.

proton shifts of H-2, H-6, and H-8 in both instances. However, the data are not sufficiently accurate to distinguish between these three positions. Enhance-

Table I. Chemical Shift Values for Purine

Position	ν_i	f_i	H, p.p.m. (relative to TMS)[a]	C^{13}, p.p.m. (relative to benzene)[a]
2	15,084,773	59,982,544	8.78	-23.1 ± 0.1
4	15,084,779	59,982,395	$(8.4 \pm 0.2)^c$	-25.9 ± 0.2^b
5	15,085,244	59,985,820	$(8.7 \pm 0.2)^c$	$+0.4 \pm 0.2^b$
6	15,084,779	59,983,005	8.86	-15.9 ± 0.1
8	15,084,778	59,982,802	8.60	-19.0 ± 0.1
H_2O			5.56^d	

[a] The practice of using positive values for lower field proton chemical shifts and higher field carbon-13 chemical shifts requires careful attention to the usage if difficulties are to be avoided. [b] Obtained from Figure 3 by calibrating the sweep rate with peaks assigned to carbons 2, 6, and 8. [c] Position of proton decoupler power which enhance peaks due to carbons 4 and 5. Calculated from ν_i, f_i, and C^{13} chemical shift obtained from sweep data. [d] Includes the labile hydrogen attached to one of the purine nitrogens.

ment through the labile hydrogens exchanging between the purine nitrogens and the aqueous solvent is not observed.

IV. Discussion

Figure 4 contains a plot of chemical shift vs. charge density as calculated for seven different quantum mechanical treatments of purine. While the scatter in this plot leaves much to be desired, a crude correlation of the data does exist for some of the treatments. Furthermore, the general correlation parallels roughly the empirical relationship proposed by Spiesecke and
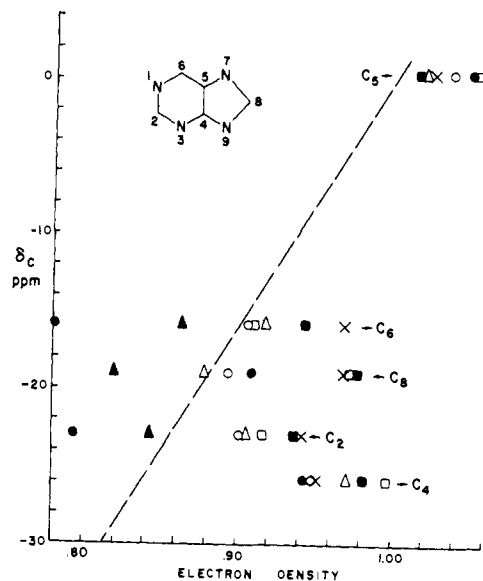

Figure 4. The C^{13} chemical shift relative to benzene is plotted vs. several theoretical estimates of the π-electron charge density. The dashed line is the empirical correlation proposed in ref. 7. Sources of calculated values are: $\square$, $\triangle$, and $\bigcirc$, ref. 11; ▲, ref. 13; ●, ref. 14; and ■ and ×, ref. 15.

Schneider[7] (dashed line in Figure 4). As observed by Lauterbur,[9] a slight negative shift due to σ-electron polarization may explain the chemical shifts for carbons centered between two nitrogen atoms. This effect, if real, would appear to be amplified in the case of the bridge head carbon C-4. As the disagreement between the several theoretical estimates is as great as the variation between these values and the empirical values of Spiesecke and Schneider, no preference is made for one approximation over another. The appearance of purine C^{13} chemical shifts in the spectral region expected for aromatic hydrocarbons with corresponding π-electron densities is felt to be more than fortuitous and supports the proposition that the distribution of π-electrons dominates the magnitude of the chemical shift parameter. σ-Bond effects while not negligible appear to be less important.